# URINE CONCENTRATIONS OF HIGH-SENSITIVITY CARDIAC TROPONIN I IN HEALTHY ADULTS – PRELIMINARY REFERENCE INTERVALS

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**Recent studies have shown the presence of troponin molecules in urine, with kidneys considered the main organs of elimination. Availability of the new generation of high-sensitivity assays has enabled detection of low concentrations of circulating cardiac troponins, but the high-sensitivity troponin assays are not designed for analysis of urine specimens. The aim of this study was to establish preliminary reference intervals for the high-sensitivity cardiac troponin I (hs-cTnI) concentrations in urine of healthy adults in Croatia. A total of 60 reference persons were selected (30 males and 30 females) and the concentrations of hs-cTnI in random urine samples were determined on the Abbott Architect i1000SR (Abbott Laboratories) analytical system with chemiluminescent immunochemical method on microparticles (CMIA, ARCHITECT STAT High Sensitive Troponin-I), accredited according to the HR EN ISO 15189:2012 standard (Medical laboratorieds – requirements for quality and competence. Geneva, International Organization for Standardization, 2012). We determined the limit of detection, total laboratory precision, expanded measurement uncertainty and preliminary estimates of the gender-specific 99th percentile of the upper reference limit (URL) using nonparametric analysis (methods). The male and female 99th percentile cut-off values were 39.3 and 35.2pg/mL, respectively. The results of this pilot study suggest that troponin I is removed from the blood by the kidneys and can be determined in the urine with CMIA, ARCHITECT STAT High Sensitive Troponin-I assays. Further research is focused on detailed studies of biochemistry and determination of troponin I in the urine as a new biological marker.**

*Key words:* troponin I, determination in urine

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## INTRODUCTION

Troponin is a protein molecule that makes the troponin complex. The troponin complex is made up of three types of troponin: troponin I, troponin T and troponin C. Troponin T is part of the troponin complex linked to tropomyosin, troponin I is an ATP-ase activity inhibitor and prevents ATP consumption, while troponin C is part of the complex to which calcium ($Ca^{2+}$) is attached (1). Troponin I has a molecular weight of about 22.5 kDa (2). There are numerous conditions that can increase troponin (myocardial infarction, pulmonary embolism, cardiac damage), but they all are commonly associated with cardiovascular damage (cardiomyocyte necrosis) (3). Troponin grows 2-3 hours after heart failure, reaching maximal value approximately 24 hours of the damage and remaining elevated for up to 8 days (4).

Within heart cells, daily exchanges of old proteins with new ones, including troponin, occur. There is also a natural daily loss of cardiac cells. These facts are one of the reasons why troponin molecules are present in the blood of healthy population. These concentrations are not large and range around 0.1-0.2 ng/L (5). While the synthesis and presence of troponin molecules in the blood is well known today, the method of secretion from the blood is still relatively unknown. Several research papers have recently tried to explain how it is removed from the blood. Some data indicate that troponin is removed in the reticuloendothelial system where it is cleaved into smaller fragments (5). There are also studies showing that enzymes such as caspases and calpain cleave the troponin molecule into small fragments that are then removed from the circulation (4). In the literature, so far there are only two statements about the presence of troponin in the urine (7,8). The first one shows that it is possible to prove troponin T and troponin I, while the second one is a summary dealing only with troponin I (7,8).

The hypothesis of this pilot study was that troponin detection in urine would be more appropriate for troponin I because it breaks in blood into smaller molecules and the likelihood of detection of these smaller immunoreactive particles is higher than for troponin T (9).

The primary purpose of this study was to confirm the presence of troponin I molecule in urine. The secondary aim was to establish preliminary reference intervals for the highly sensitive cardiac troponin I (hs-cTnI) concentrations in (first morning or randomly collected) urine of healthy adults in Croatia and to assess its potential as a new biological marker.

## PATIENTS AND METHODS

The reference interval of hs-cTnI in urine was determined in the Department of Medical Biochemistry and Laboratory Medicine, Merkur University Hospital, Zagreb, Reference Center of the Ministry of Health of the Republic of Croatia for the production of reference values in the field of general medical biochemistry on a preliminary sample of 60 healthy subjects (30 men and 30 women). The reference persons were selected using the following criteria: non-smoker aged 25-65, body weight index <30 kg/m$^2$, absence of acute and chronic diseases (heart disease and thyroid disease, hypertension, hyperlipidemia), and avoiding more severe physical activity for the last 7 days and night work during the last 30 days. All participants signed their informed consent for participation in the research.

The concentrations of hs-cTnI in random urine samples were determined on the Abbott Architect i1000SR (Abbott Laboratories) analytical system with chemiluminescent immunochemical method on microparticles (CMIA, ARCHITECT STAT High Sensitive Troponin-I), accredited according to the HR EN ISO 15189:2012 norm (10). According to the manufacturer's insert, this assay uses a sample volume of 160 μL. In the first step, cTnI present in the sample binds to the specific mouse monoclonal anti-cTnI (capture) antibody coated microparticles, the epitope binding specificity of which is directed against amino acids 24-40 on the TnI protein. In the second step, a specific mouse-human chimeric, monoclonal anti-troponin I acridinium-labeled antibody acts as a detection antibody. This chimeric antibody, which has an epitope binding specificity directed against amino acids 41-49 on the cTnI molecule, was designed to minimize the susceptibility to interferences by heterophilic antibodies (11). The method is traceable to NIST SRM 2921 and is linear in the range of 1.2 ng/L (detection limit) to 50,000 ng/L. The limit of quantification (LoQ) declared by the manufacturer is 10.0 ng/L, but there is a possible range of quantification limit of 4.0-10.0 ng/L, depending on the combination of Lot reagents and series of analyzers.

The LoQ was determined according to the CLSI EP17-A2 (11), precision was assessed based on CLSI EP15-A2 guidelines (12), and linearity was confirmed by the multicalibration curve. Expanded measurement uncertainty was estimated based on the calculated within-laboratory precision and measurement uncertainty of calibrators. The 99$^{th}$ percentile upper reference limit of the interval was calculated by nonparametric statistical analysis, according to the CLSI C28-A3 guidelines (13), using the MedCalc ver. 10.0.2.0 statistical program.

## RESULTS

Total laboratory precision was calculated according to the CLSI EP15-A2 guidelines (12) and was 2.23% (coefficient of variation, CV) in the normal range and 1.45% (CV) in the pathological area. The linearity of the methods in the declared area is confirmed by the multicalibration curve. The LoD is the lowest detectable cardiac troponin concentration reliably distinguished from the highest cardiac troponin concentration expected to be found when replicates of a sample containing the zero calibrator for a cardiac troponin assay are tested in a sample containing a low cardiac troponin concentration that can confidently be reported for clinical use. The quantification limit was determined according to the CLSI EP17-A2 guidelines (11)

and with the precision criteria for the highly sensitive test (CV <10%) (2). LoQ was verified at the concentration of 4.71 ng/L with CV% 8.41% (15). The expanded measurement uncertainty (k=2) is calculated from the total laboratory precision and calibration uncertainty and is ±4.5% for the normal range and ±2.9% for the pathological area.

Demographic characteristics and hs-cTnI concentrations in random urine samples of the 60 reference persons (30 males and 30 females) are shown in Table 1. The hs-cTnI concentration was detectable in 59 (98.3%) and quantified in 51 (85.0%) random urine samples from the selected reference population, ranging from 0.3 to 39.3 ng/L. The measured values of TnI in urine were above the detection limit (1.2 ng/L) in 59 (98.3%) subjects and above the quantification limit (4.7 ng/L) in 51 (85.0%) subjects.

The 99$^{th}$ percentile of the upper limit of the reference interval was calculated by nonparametric statistical analysis, in accordance with the CLSI C28-A3 guidelines (13), using the MedCalc ver. 10.0.2.0 statistical program (Table 2). The 99$^{th}$ percentile of the upper limit of the reference interval was 39.3 ng/L for males and 35.2 ng/L for females.

Table 1.
*Demographic characteristics and hs-cTnI concentrations in random urine samples of the selected reference persons*

| Subject number | Gender | Age (years) | Height (cm) | Weight (kg) | Body mass index (kg/m$^2$) | hs-cTnI (ng/L) |
|---|---|---|---|---|---|---|
| 1 | Female | 25 | 153 | 50 | 21.4 | 14.4 |
| 2 | Female | 31 | 158 | 50 | 20 | 19.3 |
| 3 | Female | 39 | 168 | 54 | 19.1 | 8.0 |
| 4 | Female | 25 | 164 | 50 | 18.6 | 29.7 |
| 5 | Female | 25 | 166 | 58.5 | 21.2 | 15.9 |
| 6 | Female | 57 | 160 | 53 | 20.7 | 5.1 |
| 7 | Female | 38 | 164 | 75 | 27.9 | 20.6 |
| 8 | Female | 57 | 165 | 69 | 25.3 | 28.7 |
| 9 | Female | 44 | 161 | 62 | 23.9 | 18.9 |
| 10 | Female | 48 | 170 | 65 | 22.5 | 0.3 |
| 11 | Female | 62 | 165 | 67 | 24.6 | 15.7 |
| 12 | Female | 51 | 170 | 70 | 24.2 | 19.7 |
| 13 | Female | 30 | 170 | 68 | 23.5 | 20.8 |
| 14 | Female | 44 | 165 | 80 | 29.4 | 20.5 |
| 15 | Female | 56 | 175 | 70 | 22.9 | 6.7 |
| 16 | Female | 59 | 175 | 86 | 28.1 | 3.3 |
| 17 | Female | 49 | 170 | 60 | 20 | 20.5 |
| 18 | Female | 47 | 171 | 73 | 25 | 16.8 |
| 19 | Female | 28 | 170 | 63 | 21.8 | 7.3 |
| 20 | Female | 29 | 181 | 63 | 19.2 | 15.6 |
| 21 | Female | 27 | 182 | 65 | 19.6 | 7.3 |
| 22 | Female | 51 | 164 | 66 | 24.5 | 27.3 |
| 23 | Female | 63 | 164 | 70 | 26 | 35.2 |
| 24 | Female | 63 | 164 | 69 | 25.7 | 19.1 |
| 25 | Female | 58 | 169 | 66 | 23.1 | 30.9 |
| 26 | Female | 35 | 172 | 54 | 18.3 | 27.2 |
| 27 | Female | 42 | 176 | 78 | 25.2 | 29.9 |
| 28 | Female | 27 | 168 | 58 | 20.5 | 26.2 |
| 29 | Female | 35 | 170 | 65 | 22.5 | 14.2 |
| 30 | Female | 37 | 173 | 75 | 25.1 | 7.0 |
| 31 | Male | 32 | 192 | 105 | 28.5 | 9.9 |
| 32 | Male | 51 | 183 | 93 | 27.8 | 39.3 |
| 33 | Male | 25 | 184 | 80 | 23.6 | 16.3 |
| 34 | Male | 55 | 180 | 85 | 26.2 | 9.2 |
| 35 | Male | 55 | 190 | 95 | 26.3 | 31.6 |
| 36 | Male | 45 | 177 | 88 | 28.1 | 7.2 |
| 37 | Male | 33 | 184 | 97.5 | 28.8 | 25.8 |
| 38 | Male | 26 | 171 | 75 | 25.6 | 3.9 |
| 39 | Male | 54 | 188 | 102 | 28.9 | 23.9 |
| 40 | Male | 32 | 179 | 76 | 23.7 | 3.4 |
| 41 | Male | 28 | 190 | 109 | 30 | 8.5 |
| 42 | Male | 31 | 191 | 94 | 25.8 | 1.8 |
| 43 | Male | 25 | 176 | 73 | 23.6 | 29.3 |
| 44 | Male | 28 | 191 | 92 | 25.2 | 35.0 |
| 45 | Male | 58 | 170 | 61 | 21.1 | 36.8 |
| 46 | Male | 46 | 183 | 90 | 26.9 | 2.3 |
| 47 | Male | 28 | 185 | 83 | 24.3 | 5.8 |
| 48 | Male | 28 | 189 | 92.5 | 25.9 | 14.7 |
| 49 | Male | 28 | 205 | 118 | 28.1 | 2.7 |
| 50 | Male | 40 | 183 | 96 | 28.7 | 12.4 |
| 51 | Male | 24 | 177 | 77 | 24.6 | 38.5 |
| 52 | Male | 29 | 190 | 84 | 23.3 | 3.8 |
| 53 | Male | 29 | 178 | 84 | 26.5 | 19.1 |
| 54 | Male | 32 | 192 | 96 | 26 | 27.9 |
| 55 | Male | 40 | 181 | 89 | 27.2 | 18.9 |
| 56 | Male | 26 | 190 | 91 | 25.2 | 13.3 |
| 57 | Male | 43 | 187 | 104 | 29.7 | 2.0 |
| 58 | Male | 29 | 182 | 86 | 26 | 20.2 |
| 59 | Male | 26 | 185 | 85 | 24.8 | 38.5 |
| 60 | Male | 36 | 182 | 80 | 24.2 | 9.2 |

Table 2
*Reference intervals of hs-cTnI concentrations in random urine samples of the selected reference persons*

| Methods/Assay/ Platform | Gender | Number (n) | 99th percentile of the upper limit of the reference interval (ng/L) |
|---|---|---|---|
| ARCHITECT STAT High Sensitive Troponin-I/Abbott Architect i1000SR | Male | 30 | 39.3 |
| | Female | 30 | 35.2 |

## DISCUSSION

Clinical application of the results of the determination of cTnI concentration is very often limited if older generation methods are used because of their low analytical sensitivity. The term "high sensitivity" refers to the analytical characteristics of the same method, not to the measurement of some other form of cardiac troponin I (15). These methods must meet the two basic criteria: they must be able to reliably measure (values above the detection limit) the concentration of cTnI in at least 50% of a healthy population with a variation coefficient of <10% at the 99th percentile of the upper limit of the reference interval, which represents great improvement over older methods that detect values in <20% of healthy population (15).

The restriction of the older generation method did not allow determination of biological variability (16), which has become usable by the introduction of highly sensitive methods. Clinical application of the results of the determination of hs-cTnI has a strong influence on short-term intra-individual (CVI) and inter-individual (CVG) variability. Values for CVI (%) are 15.2-24.4 and for CVG (%) 70.5-124 (17,18). Despite the improved analytical characteristics of highly sensitive methods (analytical sensitivity and precision), there is still a problem when comparing the results of different tests due to non-standardization of the methods. The reason for this is the lack of a commutable certified reference material that would provide metrological traceability (15). Despite the use of monoclonal proteins that have contributed to better specificity and sensitivity, the interpretation of results should have in mind both posttranslational and proteolytic modifications and polymorphisms of a single nucleotide affecting the structure of the cardiac troponin I molecule and may affect its immunoreactivity in human samples (15).

## CONCLUSION

Using the chemiluminescent immunochemical method on microparticles (CMIA, ARCHITECT STAT High Sensitive Troponin-I) on the Abbott Architect i1000SR (Abbott Laboratories) analytical system, we established 39.3 ng/L for males and 35.2 ng/L for females as a preliminary 99th percentile of the upper limit of the reference interval in random urine samples of the selected reference persons from Croatia. The preliminary results obtained show that troponin I is removed from the blood *via* kidneys and can be determined in the urine by the CMIA, ARCHITECT STAT High Sensitive Troponin-I used to determine TnI in plasma.

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## SAŽETAK

### KONCENTRACIJE SRČANOG VISOKOOSJETLJIVOG TROPONINA I U MOKRAĆI ZDRAVIH OSOBA − PRELIMINARNI REFERENTNI INTERVALI

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**Cilj ovoga istraživanja bio je odrediti preliminarne referentne intervale visokoosjetljivog troponina I u mokraći zdravih osoba. Koncentracije srčanog visokoosjetljivog troponina I u slučajnim uzorcima mokraće određene su na analitičkom sustavu Abbott Architecti1000SR (Abbott Laboratories) kemiluminiscentnom imunokemijskom metodom na mikročesticama (CMIA, ARCHITECT STAT High Sensitive Troponin-I), akreditiranom prema HR EN ISO 15189. Referentni interval izrađen je u Referentnom centru Ministarstva zdravstva Republike Hrvatske za izradu referentnih vrijednosti u području opće medicinske biokemije na uzorku od 30 referentnih osoba po spolu sljedećih karakteristika: nepušač, 25-65 godina, indeks tjelesne mase <30 kg/m², odsutnost akutne i kronične bolesti, bez noćnog rada tijekom zadnjih 30 dana. Granica kvantifikacija određena je sukladno smjernicama CLSI EP17-A2 i uz zadovoljavanje kriterija za preciznost za visokoosjetljivi test (KV<10 %). Linearnost metode u deklariranom području potvrđena je multikalibracijskom krivuljom. Ukupna laboratorijska preciznost izračunata je sukladno smjernicama CLSI EP15-A2 i iznosi 2,23 % (KV) u normalnom području i 1,45% (KV) u patološkom području. Proširena mjerna nesigurnost (k=2) izračunata je iz ukupne laboratorijske preciznosti i mjerne nesigurnosti kalibratora i iznosi ±4,5 % za normalno područje i ±2,9 % za patološko područje; 99. percentila gornje granice referentnog intervala izračunata je neparametarskom statističkom analizom, sukladno smjernicama CLSI C28-A3. Određena 99. percentila gornje granice referentnog intervala za visokoosjetljivi troponin I u mokraći iznosi 39,3 ng/L za muškarce i 35,2 ng/L za žene. Dobiveni preliminarni rezultati ukazuju na to da se troponin I odstranjuje iz krvi putem bubrega i da se može pouzdano mjeriti na analitičkom sustavu Abbott Architecti1000SR (Abbott Laboratories) kemiluminiscentnom imunokemijskom metodom na mikročesticama.**

*Ključne riječi:* troponin I, određivanje u mokraći